

We claim:

1. A method for determining DNA methylation patterns at cytosine sites, comprising
the steps of:

(a) obtaining genomic DNA from a DNA sample to be assayed;

5 (b) reacting the genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged to provide primers specific for the bisulfite-converted genomic sample for top strand or bottom strand methylation analysis;

10 (c) performing a PCR amplification procedure using the top strand or bottom strand specific primers;

(d) isolating the PCR amplification products;

15 (e) performing a primer extension reaction using Ms-SNuPE primers, [³²P]dNTPs and *Taq* polymerase, wherein the Ms-SNuPE primers comprise a from about a 15 mer to about a 22 mer length primer that terminates immediately 5' of a single nucleotide to be assayed; and

19 (f) determining the relative amount of allelic expression of CpG methylated sites by measuring the incorporation of different ³²P-labeled dNTPs.

2. The method of claim 1 wherein the [³²P]dNTP for top strand analysis is [³²P]dCTP or [³²P]TTP.

3. The method of claim 1 wherein the [³²P]dNTP for bottom strand analysis is [³²P]dATP or [³²P]dGTP.

4. The method of claim 1 wherein the isolation step of the PCR products uses an electrophoresis technique.

5. The method of claim 4 wherein the electrophoresis technique uses an agarose gel.

6. The method of claim 1 wherein the Ms-SNuPE primer sequence comprises a

25 sequence of at least fifteen but no more than twenty five bases having a sequence selected from the group consisting of GaL1 [SEQ ID NO. 1], GaL2 [SEQ ID NO. 2], GaL4 [SEQ ID NO. 3], HuN1 [SEQ ID NO. 5], HuN2 [SEQ ID NO. 6], HuN3 [SEQ ID NO. 7], HuN4 [SEQ ID NO. 8], HuN5 [SEQ ID NO. 8], HuN6 [SEQ ID NO. 9], CaS1 [SEQ ID NO. 10], CaS2 [SEQ ID NO. 11], CaS4 [SEQ ID NO. 12], and combinations thereof.

30 7. A Ms-SNuPE primer sequence designed to anneal to and terminate immediately 5' of a desired cytosine codon in a CpG target site ,comprising an oligonucleotide sequence of at least 15 base pairs and corresponding to a gene sequence located immediately 5' upstream from the CpG island that is frequently hypermethylated in promoter regions of somatic genes in malignant tissue.

35 8. The Ms-SNuPE primer sequence wherein the primer sequence is from about 15 to about 25 base pairs in length and selected from the group consisting of GaL1 [SEQ ID NO. 1],

GaL2 [SEQ ID NO. 2], GaL4 [SEQ ID NO. 3], HuN1 [SEQ ID NO. 5], HuN2 [SEQ ID NO. 6],
HuN3 [SEQ ID NO. 7], HuN4 [SEQ ID NO. 8], HuN5 [SEQ ID NO. 8], HuN6 [SEQ ID NO. 9],
CaS1 [SEQ ID NO. 10], CaS2 [SEQ ID NO. 11], CaS4 [SEQ ID NO. 12], and combinations
thereof.

5 9. A method for obtaining a Ms-SNuPE primer sequence designed to anneal to and
terminate immediately 5' of a desired cytosine codon in the CpG target site, comprising finding a
hypermethylated CpG island in a somatic gene from a malignant tissue or cell culture, determining
the sequence located immediately 5' upstream from the hypermethylated CpG island, and isolating
a 15 to 25 mer sequence 5' upstream from the hypermethylated CpG island for use as a Ms-SNuPE
10 primer.

15 10. A Ms-SNuPE primer comprising a 15 to 25 mer oligonucleotide sequence obtained
by the process comprising, finding a hypermethylated CpG island in a somatic gene from a
malignant tissue or cell culture, determining the sequence located immediately 5' upstream from
the hypermethylated CpG island, and isolating a 15 to 25 mer sequence 5' upstream from the
hypermethylated CpG island for use as a Ms-SNuPE primer.